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Common variable immunodeficiency is associated with polymorphic markers in the human major histocompatibility complex

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SUMMARY

Common variable immunodeficiency (CVI) is a heterogeneous condition characterized by arrest in B cell differentiation. A high frequency of null alleles of the C4 gene has been reported in patients with this disorder. We investigated the restriction fragment length polymorphisms (RFLP) of the MHC class II genes HLA-DRB, DQA, and DQB, the class III gene C4 and the tumour necrosis factor-alpha (TNF-α) gene in 40 Caucasian patients. The results showed an increase in HLA-DR3 in patients (40% *vs* 30·5%), but, more significantly, there was a striking increase in the number of CVI patients who carried a deletion of the C4A gene (46% *vs* 25%). In both patients and controls there was strong allelic association between HLA-DR3 and C4A deletion, and HLA-DR3 and TNF-α. Our results suggest that genes present on an extended haplotype containing these three polymorphisms contribute to genetic susceptibility to CVI.

Keywords immunodeficiency HLA-D C4

INTRODUCTION

Common variable immunodeficiency (CVI) is the most common form of immunodeficiency syndrome, with a prevalence of ≈2/10 000 of the UK population. The condition is characterized by low serum levels of immunoglobulins, usually with a very low IgG and IgA, but variable IgM levels. The disease can begin at any age, although the onset is usually before the age of 25 years. The main symptoms are chronic bronchitis and sinusitis due to recurrent infections with non-typable *Haemophilus influenzae* (Asherson & Webster, 1980). Friedman *et al.* (1977) were the first to show that genetic factors may be important by finding an increased incidence of autoantibodies in the serum of first degree relatives of CVI patients. Subsequently, there have been a few reports of CVI occurring in first-degree relatives, and we are aware of three such families in the UK; a mother and her son were included in the cohort described here. CVI patients usually have normal numbers of circulating B lymphocytes which fail to differentiate *in vivo* into immunoglobulin-secreting plasma cells. This block can be overcome *in vitro* in about half the patients, when the circulating B cells can be stimulated to produce IgM or both IgM and IgG with interleukin-2 (Bryant *et al.* 1990). About 20% of patients also have an obvious *in vitro* T cell defect with depressed or absent DNA synthesis following stimulation with mitogens or anti-CD3 antibodies (reviewed by Spickett, Webster & Farrant, 1990). Although chronic viral infection has been considered as a trigger for the disease, the evidence is weak (Spickett *et al.*, 1988).

A significant number of patients with selective IgA deficiency and CVI possessed rare alleles at the C2 locus or deletions in their C4A and 21-hydroxylase A (CYP21A) genes (Hammarstrom & Smith, 1983; Oen, Petty & Schroeder, 1982; Shaffer *et al.*, 1989). This deletion was found on the background of an MHC haplotype—HLA-A1, Cw7, B8, DR3—which normally occurs with a frequency of approximately 5% in the Caucasian population (Baur & Danilovs, 1980). These findings prompted us to examine the MHC genetic polymorphism in a larger group of well-defined Caucasian patients with CVI, to determine the primary MHC gene which is associated with the disease. We studied the restriction fragment length polymorphism (RFLP) of the class II genes: HLA-DR, DQA, DQB, the class III gene C4, and the tumour necrosis factor-alpha (TNF-α) gene. Our results showed that HLA-DR3, C4A deletion, and a TNF-α 5·5-kb *Nco* I fragment is commonly found in this group of patients.

SUBJECTS AND METHODS

Patients and control subjects

Forty Caucasian patients with CVI were studied (mean age 37 years, range 17–78 years). All patients had low immunoglobulin levels at diagnosis. Thirty-seven had a serum IgG level below 3·0

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g/l, and two between 3 and 5 g/l. Thirty-seven had serum IgA levels less than 0·2 g/l, with only one patient having a normal IgA level. There was one patient with selective IgA deficiency, with normal IgG and IgM levels but a failure to make IgG antibodies after immunization. IgM levels were more variable, with 34 patients having depressed levels below 0·5 g/l (28 having $<0{\cdot}2$ g/l); one patient had elevated IgM (7·7 g/l). The mean age at onset was 14 years, range 1·4–58 years, and the sex distribution was 19 women and 21 men. One hundred normal subjects served as the control population. All were Caucasian. DNA was extracted from 20 ml of peripheral blood by standard methods.

MHC class II typing

HLA-DR, DQA and DQB typing was performed by RFLP analysis of Southern blots of *Taq*I digested genomic DNA from patients and controls. The DRB probe is a 520-bp *Pst*I fragment of a full-length cDNA clone of the HLA-DR4DRB1 gene, which encodes the $\beta 2$ and transmembrane domains of DRβ. The DQA probe is a full-length cDNA clone of the DQA gene from the same cell line. The DQB probe is a full-length cDNA clone of the DQB gene.

C4 RFLP

This was performed using a 500-bp *BamH*I to *Kpn*I genomic fragment probe which spans the 5′ end of the C4 gene (Schneider *et al.*, 1986). DNA samples were digested with *Taq*I.

TNF RFLP

This was analysed using a 2·9-kb *Eco*RI genomic fragment which spans the TNF-α gene (gift of Dr Duncan Campbell) to hybridize Southern blots digested with *Nco*I.

Southern blotting

Seven micrograms of genomic DNA from each subject were digested with restriction endonuclease in the recommended buffer. Southern transfer was performed using standard techniques on to nylon membranes (Hybond-N, Amersham International, Amersham, UK). Filters were prehybridized in $6\times$SSC, 0·1% SDS, $10\times$ Denhardt's, 10% dextran sulphate and 50 μg/ml sheared salmon sperm DNA for >2 h at 65°C, then hybridized with the radiolabelled probe (1×10^6 ct/min/ml of hybridization buffer) overnight. Filters were washed to high stringency, the final wash being 30 min at 65°C in $0{\cdot}2\times$SSC, 0·1% SDS.

Statistical analysis

This was performed using the χ^2 statistic with Yates' correction.

RESULTS

DR antigen frequency

DR polymorphism was analysed by RFLP of HLA-DRB and DQA genes in the patient and control groups, using the characteristic RFLP fragments generated by each probe to define the HLA-DR genotype for each individual (Bidwell, 1988). The results are summarized in Table 1. This showed an increased frequency of HLA-DR3 in the patient population (40% *vs* 30·5%). The increase did not reach statistical significance after correction for the number of antigens analysed ($\chi^2=3{\cdot}54$). None of the other antigens were significantly different in frequency. The frequencies of DQA and DQB RFLPs were not significantly different in the two groups (data not shown).

Table 1. HLA-DR antigen frequency in CVI patients and normal subjects

DR	Patients ($n=40$)	%	Controls ($n=95$)	%
1	8	20	18	18·9
2	7	17·5	24	25·3
3	19	40	29	30·5*
4	13	32·5	40	42·1
5	7	17·5	14	14·7
w6	5	12·5	17	17·8
7	8	20	18	18·9
8	0	—	4	4·2
10	1	2·5	0	—
χ	0	—	12	12·6

HLA-DR phenotype was assigned by RFLP (see text for details).

* $\chi^2=2{\cdot}84$, $P>0{\cdot}05$.

Table 2. Frequency of C4A gene deletions in patients and normal controls

	C4A deletions	
	+	−
Patients ($n=39$)	18 (46%)	21 (54%)
Controls ($n=100$)	25 (25%)	75 (75%)

+ indicates individuals who possess the deletion as defined by RFLP of the C4 genes. − indicates absence of the deletion.

$\chi^2=4{\cdot}93$, $P<0{\cdot}05$.

Deletions of the C4A gene

RFLP analysis of the C4 gene was used to determine the gene frequency of this deletion in patients and the normal subjects. Instead of the common 7·0-kb fragment, which represents the normal C4A gene, deletion of part of the gene results in a 6·4-kb fragment. The frequency of this fragment was increased significantly in patients with CVI (46% *vs* 25%) (Table 2). This deletion is strongly associated with the presence of HLA-DR3, as 16 of 18 patients who were HLA-DR3 also possessed this deletion, and only two of 21 who were non-DR3 carried it (Table 3).

TNF RFLP

A di-allelic RFLP of the TNF-α gene was identified using the restriction enzyme *Nco*I. The two fragments are 10·5 kb and 5·5 kb in size. The gene frequencies of the two alleles in the patient population were 59% and 41% respectively. The

Table 3. HLA-DR3 is strongly associated with C4A gene deletion

	C4A deletion	
	+	−
DR3$^+$	16	2
DR3$^-$	2	19

$\chi^2 = 21{\cdot}5$, $P < 0{\cdot}0005$.

Table 4. Allelic association between HLA-DR3 and TNF-α RFLP

	TNF (10·5 kb) upper*		TNF (5·5 kb) lower†	
	+	−	+	−
DR3$^+$	12	6	15	3
DR3$^-$	19	2	9	12

* $\chi^2 = 2{\cdot}07$, $P > 0{\cdot}05$.
† $\chi^2 = 5{\cdot}11$, $P < 0{\cdot}025$.

possession of the 5·5-kb fragment was significantly associated with the individual also possessing HLA-DR3, as seen from the results in Table 4. Fifteen out of 18 DR3-positive patients had at least one lower 5·5-kb fragment compared with nine out of 21 DR3-negative patients. This was statistically significant ($\chi^2 = 5{\cdot}1$, $P < 0{\cdot}025$) (Table 4).

DISCUSSION

An association between MHC genes and humoral immunodeficiency was initially described in patients with IgA deficiency, and was extended in a more recent study of 19 patients with CVI, which reported that HLA-DR3 was increased in frequency (Shaffer *et al.*, 1989). As there is strong linkage disequilibrium between polymorphisms at different genetic loci within the MHC, we investigated the polymorphisms of genes which map centromeric and telomeric to HLA-DR. The C4 gene lies in the class III region of the MHC, and the TNF gene between C2 and HLA-B, approximately 600 kb centromeric to the HLA-B locus (Sargent *et al.*, 1989; Spies *et al.*, 1989). We have studied the DNA polymorphism of these genes in a large group of patients with CVI by RFLP techniques, and have shown an increased frequency of HLA-DR3 and deletions affecting the C4A gene in the patient group.

There was a significantly increased frequency of deletions of the C4A gene in the patients. In the general population, this deletion is found most frequently as part of an extended haplotype which contains HLA-DR3 and HLA-B8 (Schneider *et al.*, 1986), and in patients with systemic lupus erythematosus we have shown that this C4A deletion is almost always found on the HLA-B8, -DR3, Bf*S, C2*C, C4AQ*0, C4B*1 haplotype (So *et al.*, 1990). The increased frequency of HLA-DR3, and its allelic association with C4A deletion and the TNF-α 5·5-kb *Nco* I fragment in our patients, suggests that the frequency of this extended haplotype is increased in CVI, though we have not formally demonstrated this by family studies or typing for HLA class I antigens. Recent studies of TNF-α RFLP in other patient groups have also found such an allelic association (Fugger *et al.*, 1989). Interestingly, in the mother–son pair with CVI, both carry HLA-DR3, C4A deletion and the 5·5-kb TNF-α fragment.

Because of the strong linkage disequilibrium between the different MHC loci, it is extremely difficult to differentiate whether the primary association is with the entire haplotype or a single gene within the MHC. The calculated relative risks of HLA-DR3 and C4A deletion were 1·99 and 2·57 respectively, and suggest that HLA-DR3 may be a secondary association, due to the common occurrence of the HLA-B8, C4A*Q0, DR3 haplotype in the Caucasian population. Although the cause of CVI is unknown, immunological mechanisms are likely to play a part, and autoimmune phenomena such as vitiligo and autoimmune blood dyscrasias are found more frequently in these patients. The polymorphic genes we have studied are known to have important immune functions, though the mechanisms by which they may individually influence antibody production *in vivo* is unclear. This same haplotype has been implicated in immune hypo-responsiveness to hepatitis B immunization (Alper *et al.*, 1989), in rapid decline of CD4$^+$ T cells in patients with HIV-1 infection (Kaslow *et al.*, 1990) and systemic lupus erythematosus (Fielder *et al.*, 1983). Our results suggest this haplotype contains gene(s) which confer an increased susceptibility to developing CVI.

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